STUDIES OF SALIVARY MUCIN AND ITS QUANTITATIVE DETERMINATION

BY

JAMES MASANAGA INOUYE, B.S., M.A.

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE FACULTY OF PURE SCIENCE
OF COLUMBIA UNIVERSITY

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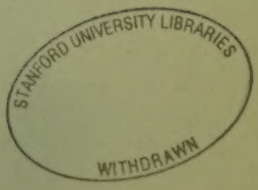

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J. M. I.

Laboratory of Biological Chemistry,
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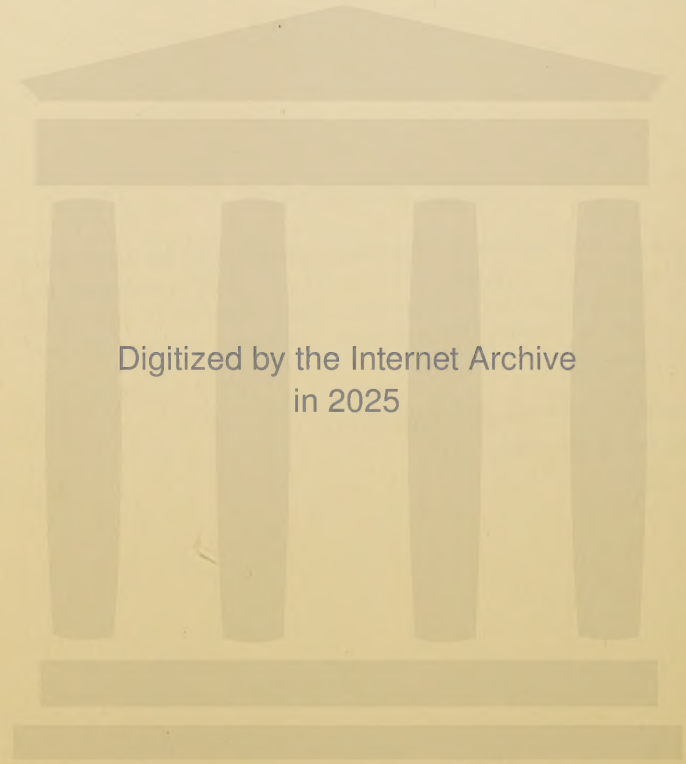
May, 1924.



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STUDIES OF SALIVARY MUCIN, AND ITS QUANTITATIVE DETERMINATION

I

Mucins are formed by various glands associated with the mucous membranes of the body. In normal conditions, mucin is important in protection and lubrication, prevention of desiccation of tissue surfaces, and in other relationships. Under various pathological conditions changes in mucin secretion are known to be important.

The knowledge of these changes is at present based merely on gross observations. In order that normal and pathological variations in mucin secretion and the clinical implications of these variations may be studied, methods are needed for the quantitative determination of mucins in biological materials.

Little is known at present about the differences among mucins formed by the different mucin-producing tissues of the body. For these studies, the mucin of the submaxillary salivary gland was chosen. The submaxillary glands of oxen provide a comparatively convenient source for the preparation of the protein. The salivary mucin is believed to be an important factor in dental and oral physiology and pathology. Through the formation of plaques on the enamel surface, with consequent entanglement of bacteria, and localization and concentration of the effects of fermentation acids, mucin is believed to bear direct causal relation to the incidence of caries.

A review of the published work showed that the available data at present on the general properties and reactions of mucin are so few and incomplete that certain qualitative studies were undertaken as a basis for the development of a quantitative method.

II

Mucin[1] was first examined by Städeler (cited by Obolensky (27). Various workers have prepared and studied mucins from

[1] In this dissertation, the mucoids, including some of the connective tissue proteins previously called mucins, are assumed to be sufficiently different from the true mucins to justify elimination of reference to them. The exact line of differentiation between the different types of glycoproteins can not be closely drawn, but we use the classification which is conventional at present.

widely different sources: Obolensky (27), Hammarsten (9), Lothrop and Gies (21) and Dejust (4) from salivary glands; Müller (25), Lothrop (21) from saliva; Hammarsten (11) and Landwehr (16) from bile; Eichwald (5), Cavalcaselle (2), and Hammarsten (8) from snail; Holst (12) from synovial fluid; Levene (19) from gastric membrane and other sources; Giacosa (6) and Schultz and Ditthorn (32) from frog spawn; Hammarsten (10) from perch eggs; Müller (27) from mucosa of the air passages; Charrin and Desgrez (3) from bacterial products; and Ishii (13) and Oshima and Tadokoro (28) from yams. The carbon, hydrogen, nitrogen, oxygen, sulphur, ash contents, etc., have been determined, and various qualitative reactions have been studied.

The hydrolytic products of the mucins have been proved or isolated and studied by Müller and Seeman (26), Steudel (33), Otori (29), Posner and Gies (31), Malenück (23), and especially by Levene (19) and his collaborators.

The relation of salivary mucin to dental caries and oral pathology has been investigated by Lohman (20), Miller (24), Gies (7), Lothrop and Gies (22), Kirk (15), Pickerill (30), and Jones (14).

The first preparation of submaxillary mucin was made by Obolensky (27) in 1870. He cleaned and washed the glands, ground them with glass, extracted overnight with water, filtered, precipitated the mucin with acetic acid, washed the precipitate with hot alcohol, and dried it. In 1881 Landwehr (16) extracted mucin from bile with strong hydrochloric acid, and precipitated it by neutralization or by dilution with water; he then redissolved and reprecipitated with dilute hydrochloric acid, washed the precipitate several times with alcohol and ether, and dried at 110° C. These methods, however, were found to be unsatisfactory. The method of extracting mucin from the tissues with weak alkali has been used by several workers. Recently, Dejust (4), prepared submaxillary salivary mucin by extracting the glands of oxen with 0.5 per cent potassium hydroxide, followed by precipitation with acetic acid. In 1888, Hammarsten (9) introduced a method, later modified by Levene (17), for the preparation of mucin from submaxillary glands which was found to

be the best method available, and this method (1), with minor modifications, we adopted, as outlined below.

III

A supply of submaxillary glands of oxen was obtained, fresh, from the slaughter house. They were freed of extraneous tissue, finely hashed, and extracted twice with distilled water overnight, using chloroform as a preservative. The extract was siphoned off and filtered through cheesecloth and silk. The filtrate was shaken with ether and allowed to stand overnight. It was then strained through silk and filtered through filter paper, giving a heavy, viscous, clear liquid. This was precipitated with dilute hydrochloric acid, to a strength of 0.03 per cent. The precipitate so obtained was redissolved in 0.15 per cent hydrochloric acid, and reprecipitated by pouring into distilled water (four volumes), with vigorous agitation. Each time the mucin chloride solution was strained through silk in order to remove particles of undissolved mucin. This purification was repeated three times, and at each precipitation the mucin separated out as characteristic stringy masses, in most cases depositing on the stirring rod, though sometimes separating out on the surface as a sticky, viscous, gummy mass after vigorous agitation. The final precipitate was washed by shaking out in a large volume of distilled water, and was then treated with freshly distilled neutral alcohol and sedimented; and this was repeated until the strength of alcohol reached 95 per cent. It was then filtered, and washed three times with ether. The sample so obtained was colorless or white as flour. There seemed in this state at least, no trace of brown color. Dried at room temperature overnight, it tended to darken very slightly. This method, while long and tedious, gave the best results.

Centrifugation could not satisfactorily be substituted for filtration because of the poor separation of tissue debris, etc., and also because of the large volumes involved. Purification by resolution in sodium hydroxide was not as satisfactory as in hydrochloric acid, since it was found that the long or repeated contact with the alkali caused denaturing of the mucin. One product prepared by precipitation from sodium hydroxide solution gave nitrogen, sulphur, and ash contents identical with those reported by Landwehr (16), but it did

not show the characteristic properties of mucin. The powder was exceedingly light, not viscous in water; upon addition of fifth normal sodium hydroxide no heavy slimy viscous sodium mucinate formed; its solution gave heavy precipitates with lead acetate.

The yield of purified mucin varied from 4.81 to 7.87 grams for each kilogram of fresh hashed gland tissue.

It may be noted that a nine pound lot of parotid salivary glands, when worked up by this method, yielded no mucin.

IV

The water soluble mucin salt for use in these experiments was prepared from the purified mucin by the method adopted by Lothrop (21), with the elimination of the use of 0.1 per cent sodium hydroxide solution, as follows:

One part of mucin was rubbed up in a mortar in small portion with 100 parts of a 0.5 per cent sodium bicarbonate solution; to the mixture were added ten volumes of 0.5 per cent sodium carbonate solution; and it was allowed to stand overnight in the cold. Large swollen particles of jelly-like masses were observed here and there. It was then squeezed through silk, which gave a whitish thick paste-like fluid. By agitating this in a large beaker, it went entirely into solution. The solution was then dialyzed in a freshly prepared collodion bag of two liter capacity, against running freshly-distilled water until acid to phenolphthalein and alkaline to litmus. This dialysis required at least eighteen hours. The mucinate solution so obtained was then precipitated by pouring into eight volumes of neutral 95 per cent alcohol. When the dialyzed mucinate was first poured into the alcohol, no appreciable amount of precipitate was observed, even after standing for thirty minutes. However, upon addition of 3 cc. of 20 per cent sodium chloride for every 2250 cc. of the alcohol-water mixture, and agitation, an opalescent, milky, and finally white heavy precipitate settled. The first fraction was collected at the end of three hours sedimentation, and the remainder at later intervals. These samples were washed with cold alcohol until free from chloride, then washed three times with ether, and dried in a desiccator to constant weight.

The sample collected as the first sedimentation fraction was not totally soluble in distilled water, but required the addition of a small

amount of sodium hydroxide solution to effect complete solution. The other samples, however, were easily and completely soluble in distilled water.

These preparations gave the expected nitrogen, sulphur and ash contents; they gave reducing material on hydrolysis; no purine base could be detected in the hydrolysate.

We also had available, through the courtesy of Professor Gies, two samples of sodium mucinate prepared by Lothrop (21), and designated in his publication as samples VI and VII. Throughout our experiments, these were compared with our own preparations.

Weighed amounts of mucinate were dissolved in distilled water. Usually, small additions of ether were made, to break the foam and to act as a preservative. When necessary, small amounts of sodium hydroxide were added to obtain complete solution (as in the case of samples VI and VII of Lothrop, which required 1.8 and 0.9 cc. of N/5 alkali, respectively, in 100 cc. of a 0.1 per cent solution of the mucinate). This addition of alkali did not make the solutions alkaline to phenolphthalein. The solutions were kept in the ice box, but were brought to 20° C. for pipetting.

V

A review of the reactions of mucin, as reported in the literature, suggested several possibilities for the separation of mucin from the other proteins occurring with it, such as the precipitation of the other proteins by ferrocyanic acid in the presence of sodium chloride, etc. Without giving the details of the negative results, it will be sufficient to say that these methods were not useful for the isolation of mucin.

One very interesting fact appeared. It is commonly stated by Hammarsten (9) and other authorities that mucin is precipitated by salts of lead and mercury. To our surprise we found that with solutions of sodium mucinate in concentrations up to 0.1 per cent, the addition of lead acetate or mercuric chloride (5 to 10 per cent solutions) did not cause precipitation of the protein. Many such tests were made. Care was taken that the reaction of the mixture was well on the alkaline side of the probable isoelectric point of the mucin. The addition of the metal caused an opalescence; or in the presence of excess alkali a discrete precipitate (of hydroxide). The

mucinate solution plus the metallic salt was filtered and the nitrogen content of the filtrate was determined. As a rule, the mucin was found in toto in the filtrate. However, in spite of the fact that variable factors such as reaction, time, concentration, etc., were carefully controlled, this could not always be relied on. Similarly, serum protein from dog blood, or ox serum in known amounts was added to known mucinate solutions and treated with the metallic salt and filtered. Again we found that while in the majority of cases the nitrogen figures indicated complete separation of the serum protein from the mucin, yet the results were not sufficiently constant to warrant the use of this method for the analysis of unknown solutions. The treatment with lead or mercury was also found to be not favorable for the purification of mucin because of technical difficulties in removing the metal. Our results, however, were sufficiently promising to suggest that these points are worthy of further careful study.

VI

Attempts were made, on the mucinate solution and on human saliva, to precipitate the mucin with hydrochloric acid or acetic acid and remove the precipitate by filtration for subsequent weighing or nitrogen determination. We found that only 50 to 80 per cent of the mucin could be obtained in this way. At the point of complete precipitation, from solutions of concentrations of biological range, the mucin separated out in the form of a practically permanent turbidity that could not be properly removed either by filtration or centrifugation.

This difficulty, while ruling out the use of the direct gravimetric method for the determination of the mucin, after acid precipitation, suggested that the conditions were such that nephelometry might be profitably used. Our problem then became the study of the conditions of this precipitation with a view to adapting it to the nephelometric determination of the relative amount of mucin precipitated from saliva by acidification.

VII

We found that for the precipitation of mucin from solutions of biological concentration, 0.02 normal hydrochloric acid was a convenient strength to use. At a P_H of from 3 to 2.7 titration with this acid gave a maximum turbidity as shown by nephelometric observa-

tions.[2] It was found that titration until an additional drop of the acid caused no visible added clouding gave an accurate end-point for the titration, as the curve of turbidity is flat at this point, and with care there is no danger of addition of sufficient excess of the acid to cause diminution of the turbidity by resolution of the mucin.

An example of such a titration is tabulated below. 20 cc. samples of a 0.1 per cent solution of sodium mucinate prepared as described above were treated with 0.02 N hydrochloric acid in increasing amounts. The final volume was kept constant with distilled water. The nephelometric readings were made between consecutive samples, and the ratio was calculated to the maximum turbidity set at 20 mm.

No.	0.02 N HCl added, cc.	Dist. water added, cc.	Nephelom. reading, mm.
1	4.5	3.0	69.6
2	5.0	2.5	33.1
3	5.5	2.0	20.7
4	6.0	1.5	20.5
5	6.25	1.25	20.0
6	6.5	1.0	20.0
7	6.75	0.75	20.8
8	7.0	0.5	28.0
9	7.5	0.0	36.7

Another example was as follows: 10 cc. samples of a 0.05 per cent solution of sodium mucinate were treated as above. The nephelometric readings are similarly recorded:

No.	0.02 N HCl added, cc.	Dist. water added, cc.	Nephelom. reading, mm.
1	0.5	1.4	46.8
2	0.7	1.2	44.7
3	0.9	1.0	39.0
4	1.1	0.9	22.3
5	1.3	0.6	20.0
6	1.5	0.4	20.0
7	1.7	0.2	20.2
8	1.9	0.0	25.5

[2] The hydrogen ion concentration was determined by means of the indicator method as described by Clark. Through the courtesy of Dr. Baird Hastings and Mr. Julius Sendroy, of the Rockefeller Institute, duplicate determinations were also made electrometrically.

Similar results were obtained with solutions of our different mucinate samples in concentration of from 0.2 to 0.025 per cent.

Different samples of the same mucinate solution, so titrated to maximum turbidity, gave the same nephelometric reading. A given mucinate solution, diluted with water in known volumes, and then titrated to maximum turbidity, gave the theoretical reading compared with the original, provided the readings differed by less than 20 per cent. A given mucinate solution compared with another preparation showed somewhat different readings in cases where the purity of the mucinate differed; but those samples which we believe to be the purest gave practically identical turbidities.

The first difficulty in applying these results to salivary analysis was the fact that normal saliva usually has a considerable turbidity, due to epithelial debris, etc. This initial turbidity must be removed before nephelometry can be used. Direct filtration or centrifugation of the saliva was tried. When filtered, however, mucin in the ordinary condition of its occurrence in saliva, does not distribute itself evenly between the filtered and the unfiltered portions. Similarly, the lower layer of a centrifuged sample contains a higher proportion of mucin than does the upper layer. (This partial separation by filtering or centrifuging may perhaps be due to some of the protein, possibly as mucin or acid salts of mucin instead of the normal mucinate, being, though highly hydrated and apparently in homogeneous solution, not in a state of as fine dispersion as the rest.)

We found that, if the saliva or mucinate solution was made rather strongly alkaline by the addition of sodium hydroxide, it could then be readily filtered clear, and an aliquot portion of the filtrate contained the theoretical amount of nitrogen. This then was adopted as the means of clearing the saliva of its initial turbidity.

The next difficulty arose from the fact that when this alkaline filtrate was treated with dilute hydrochloric acid, we obtained incomplete precipitation of the mucin. It has long been noted (Hammarstein 9) that the addition of a fairly large amount of sodium chloride (5 to 10 per cent) to a mucinate solution may completely prevent the precipitation of the mucin by acid. We took dilute solution of mucinate (0.1 and 0.05 per cent) and added sodium chloride (2 M solution) in known amounts, then precipitated the mucin with hydrochloric acid, and compared the resultant turbidities with that

produced in another sample treated in the same way but without the addition of the salt. Small concentrations of the salt in the precipitation mixtures (as low as 0.05 molar) caused an appreciable diminution in the turbidity.

The only feasible way found for obviating this difficulty was the removal of the salt by dialysis. We therefore dialyzed the alkalinized and filtered saliva or mucinate solution in a collodion bag against running water until acid to phenolphthalein, and then against several changes of distilled water. The dialyzed material could then be made up to known volume, titrated to maximum turbidity with 0.02 N hydrochloric acid, and this turbidity compared with a standard of pure mucinate solution so precipitated. The details of this procedure are outlined below. The standard may be set conveniently at 20 mm., and the concentration of the unknown may be calculated in the usual way by inverse ratio of the readings, provided these readings do not differ by more than 20 per cent. If the difference in the readings is greater than 20 per cent, another reading should be made after appropriate dilution of the standard solution or of the unknown so that the readings will more closely approximate each other.

The analytical method adopted, then, may be outlined as follows (half the quantities may be used): A 20 cc. sample of saliva is made alkaline with 5 cc. of 5 per cent sodium hydroxide solution.[1] This is thoroughly mixed, and filtered through a dry filter. An aliquot portion (20 cc.) of the filtrate is then placed in a collodion bag (made for convenience in a 100 cc. centrifuge tube) and dialyzed against running water until no longer alkaline to phenolphthalein, and then against several changes of distilled water. The time required for this dialysis is usually about five hours. The contents of the bag are then transferred to a volumetric flask of 50 cc. capacity, the washings from the bag added, and made to the mark with distilled water. A sample (10 to 20 cc.) of this is then treated with 0.02 N hydrochloric acid from a burette, drop by drop, until one additional drop fails to give more precipitate. This is then compared nephelometrically with a standard prepared as follows: 0.1 gm. of sodium mucinate is dissolved in water with the aid of a minimum amount

[1] The apparent danger of this treatment, in view of the denaturing effect of alkali on mucin, is recognized. Our results as shown later, however, indicate that, in the concentration of alkali used and for the time during which it is in contact with the mucin, no appreciable error is introduced.

of alkali, if necessary, in a 100 cc. volumetric flask, and made to the mark with distilled water. The excess alkali is then dialyzed out from a 20 cc. sample, as described above. The contents of the bag are washed into a 50 cc. volumetric flask, and made to volume with water. Ten or twenty cc. of this are then titrated with 0.02 N hydrochloric acid to maximum turbidity. If a mucinate sample is available which is completely soluble in water without the addition of any extra alkali, it may be used directly in strength of 0.05 per cent as the standard, without dialyzing.

If the nephelometric readings differ by more than 20 per cent, a new dilution of the standard or of the unknown should be made to bring the readings more closely together.

If the dialyzed salivary mixture shows an appreciable turbidity before addition of the acid (due to globulin precipitation), this turbidity may be estimated in terms of the standard, and deducted as a correction in the final calculation.

The concentration of mucinate in the saliva may be calculated by the following formula:

$$\text{mucinate content of saliva} = \frac{\text{neph. reading of standard}}{\text{neph. reading of unknown}} \times 3.125 \times \text{concn. of standard.}$$

VIII

Because of the great difficulty in preparing pure sodium mucinate to be used as a standard, it was desired to find, if possible, some artificial standard which would give a comparable turbidity in the nephelometer. Very many things were tried, such as silver chloride with a stabilizing colloid, precipitated chalk, aluminum hydroxide, calcium stearate, and others. But it was found that the tint and texture of these turbidities was such that they could not be matched with the mucin in the nephelometer. Finally, kaolin suspended in a 5 per cent solution of gum acacia was found to give a turbidity altogether comparable with that of mucin precipitated under our conditions. The finer kaolin was obtained by shaking 50 grams of kaolin in 1000 cc. of distilled water in a graduated cylinder, sedimenting for five minutes, and then decanting the top 50 cc. of the liquid. This was added in equal amount to a 10 per cent solution of

gum acacia U. S. P. in water, and diluted with 5 per cent acacia until it gave the same turbidity as the precipitated mucinate solution. This artificial standard may be kept for some time without change, thus avoiding the repeated preparation of pure mucinate solution. This standard is purely empirical, and is, we realize, far from ideal, but it is the best that can be offered at present, and may be of considerable aid in the use of the method for clinical analysis.

IX

Human saliva was collected, the flow being stimulated by chewing pure paraffin. Samples were analyzed by this method, and the mucin content calculated. Pure sodium mucinate was added, and the mixtures were again analyzed to determine the percentage recovery of the added mucinate. We found that the recovery could be made with an error of less than 4 per cent. Examples of such analyses are given below.

Sample of saliva, cc.	Mucinate in saliva, %	Mucinate in saliva, gm.	Mucinate added, gm.	Total gm. theoret.	Mucinate found, gm.	Diff. gm.	Error in recovery %
20	0.127	0.0254	0.0050	0.0304	0.0303	0.0001	−2
20	0.146	0.0293	0.0050	0.0343	0.0341	0.0002	−4
25	0.077	0.0192	0.0113	0.0305	0.0302	0.0003	−2.6
20	0.281	0.0562	0.0150	0.0712	0.0708	0.0004	−3.3
20	0.155	0.0310	0.0200	0.0510	0.0508	0.0002	−1
20	0.100	0.0201	0.0200	0.0401	0.0403	0.0002	+1

A further check was made by the addition of small amounts of serum protein. This did not change the analytical results for the mucin.

X

SUMMARY

Mucin from submaxillary glands and sodium mucinate were prepared by standard methods, with minor modifications. The resulting mucinate powder was totally soluble in water.

Mucin is said to be precipitated by lead and mercury; we find that in the biological range of concentration this does not occur.

The reaction of maximum precipitation of mucin by dilute acid is P_H 3 to 2.7. This point of maximum precipitation can be accurately obtained for practical purposes by titration with fiftieth normal hydrochloric acid, using as an end point the lack of increase in the cloud on the addition of a drop of the acid.

Various methods of determining mucin were tried. The nature of the turbidity produced by acid precipitation, while unsuited for direct gravimetric determination after separation of the mucin, suggested the use of the nephelometer.

A nephelometric method for the determination of mucin is proposed, with preliminary clearing of the normally turbid saliva, and removal of interfering salts by dialysis.

An artificial standard is described, which may be used in the practical clinical application of the method.

Added mucin can be recovered from saliva by this method with an error of less than 4 per cent.

These studies apply to mucin solution of the concentration commonly found in saliva.

We believe that this method may be used for salivary analysis in the study of the relation of mucin content to the physiology and pathology of the mouth, with the promise of yielding important data on the subject.

BIBLIOGRAPHY

1. Abderhalden: *Hnbch. d. biochem. Arbeitsmethoden,* vol. ii, 410.
2. Cavalcaselle: *Zentr. biochem. und biophys.,* 1911, xi, 866.
3. Charrin and Desgrez: *Compt. rend. soc. biolog.,* 1898, 1, 209.
4. Dejust: *Bul. de la Soc. de Chim. Biol.,* 1920, ii, 208.
5. Eichwald: *Liebig's Ann. d. Chem. und Pharmac.,* 1865, 175.
6. Giacosa: *Zeit. f. physiol. Chem.,* 1882, vii, 40.
7. Gies: *Jour. of Allied (Dental) Soc.,* 1912, vii, 397.
8. Hammarsten: *Pflüger's Arch. f. Physiol.,* 1885, xxxvi, 373.
9. Hammarsten: *Zeit. f. physiol. Chem.,* 1888, xii, 163.
10. Hammarsten: *Skand. Arch. f. Physiol.,* 1905, xvii, 113.
11. Hammarsten: *Ergebn. d. Physiol.,* 1905, iv, 1.
12. Holst: *Zeit. f. physiol. Chem.,* 1904, xlv, 145.
13. Ishii: *Bull. Coll. Agr. Imp. Univ. Japan,* ii, No. 2 (*Jahresber. d. Thierchem.,* 1894, xxiv, 29).
14. Jones: *Dental Review,* 1911, xxv, 1167.
15. Kirk: *Dental Cosmos,* 1910, lii, 729.
16. Landwehr: *Zeit. f. physiol. Chem.,* 1881, v. 371.
17. Levene: *Zeit. f. physiol. Chem.,* 1901, xxxi, 395.
18. Levene: *Journ. of Biol. Chem.,* 1918, xxvi, 105.
19. Levene: *Monograph No. 18, Rockefeller Institute for Med. Research* (1923).
20. Lohmann: *Arch. f. Zahnheilkunde,* 1904.
21. Lothrop: *Journ. of Allied (Dental) Soc.,* 1912, vii, 410.
22. Lothrop and Gies: *Journ. of Allied (Dental) Soc.,* 1910, v, 269.
23. Malenück: *Biochem. Centr.,* 1904, iii, 223.
24. Miller: *Dental Cosmos,* 1904, xlvi, 986, 1086, and 1905, xlvii, 1293.
25. Müller: *Zeit. f. Biol.,* 1901, xlii, 561.
26. Müller and Seeman: *Deutsch. med. Wochenschr.,* 1899, xxv., 209.
27. Obolensky: *Med. chem. Untersuch.,* 1871, 590.
28. Oshima and Tadokoro: *Jour. Coll. Agr., Tohoku Imp. Univ., Japan,* vi, 243 (*Chemical Abstracts,* 1912, vi, 1172).
29. Otori: *Zeit. f. physiol. Chem.,* 1904, xlii, 453.
30. Pickerill: *Prevention of Dental Caries,* 1912, p. 181.
31. Posner and Gies: *Amer. Journ. of Physiol.,* 1904, xi, 404.
32. Schultz and Ditthorn: *Zeit. f. physiol. Chem.,* 1900, xxix, 373, and 1901, xxxii, 428.
33. Steudel: *Zeit. f. physiol. Chem.,* 1901, xxxiv, 353.

VITA

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